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INTEGRATED VIRUS DETECTION SYSTEM CHARACTERIZATION OF MS2 AND TBSV AFTER PULSED LAMP EXPOSURE

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14. ABSTRACT
The ability to decontaminate microorganisms with a broad band pulsed light system has been demonstrated (ERDEC-TR-456). This study will allow the effects of the pulsed lamp decontamination effects on MS2 bacteriophage and tomato bushy stunt virus to be analyzed quickly without historical microbiological techniques. The Integrated Virus Detection System can analyze multiple samples quickly and allows rapid determination of pulsed lamp effects.

15. SUBJECT TERMS

Virus detection	Differential mobility analyzer	Condensation particle counter
Biological agents	Electrospray	Pulsed Lamp
Decontamination	MS2	Broad band spectrum

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PREFACE

The work deseribed in this report was started in January 2005 and completed in September 2005.

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INTEGRATED VIRUS DETECTION SYSTEM CHARACTERIZATION OF MS2 AND TBSV AFTER PULSED LAMP EXPOSURE

1. INTRODUCTION

This report will examine the effects of MS2 bacteriophage and tomato bushy stunt virus (TBSV) exposure to filtered and unfiltered pulsed lamp exposure. Various filters will be placed in the path of the lamp to determine effects of wavelength on the decontamination of MS2 and TBSV. Sample virus content will be analyzed with the Integrated Virus Detector System (IVDS) before and after exposure to the pulsed light.

2. EXPERIMENTAL SETUP

2.1 Pulsed Lamp Setup

The pulsed lamp is housed in a stainless steel enclosure, 24 in. x 18 in. x 20 in., centered on the inner top panel, parallel to the opening. The enclosure is interlocked to disarm the control panel in the event the door is either open or opens during experimentation. Adjacent to the enclosure is the electronics for activating the lamp. The control panel allows the repetition rate to be set from 1 to 9 pulses. More pulses can be activated by reactivating the start button. Samples were centered under the lamp in the enclosure. A filter hood was used in all experiments to allow the placement of various filters in the light path to examine the effects of different wavelengths. The filter hood schematic and distance from the lamp is shown in Figure 1. The virus samples were exposed in a 6 well microscope slide. The slide, shown in Figure 2, is 2 in. x 3 in. x ¼ in. thick. The wells are 3/64 in. deep.

2.2 Filter Information

Various filters were used to attenuate the energy or wavelength from the pulsed lamp before impingement on the virus samples. The filters were measured by the U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) to determine actual wavelength cutoffs for each filter. The filter measurements are shown in Figures 3, 4, 5, and 7. Large and small Petri dishes, the area of the Petri dish with the logo (Figure 6), and the well sample holder (Figure 8) were also measured. The Petri dishes all allow transmittance in the visible, while blocking the UV below 310-325 nm at 50% transmittance (T). The Petri dishes should be uncovered when used in subsequent experiments for pulsed light effects. The well slide blocks the UV below 325 nm at 50%T, and there should not be a large effect from secondary bounces from the stainless surface below the slide.

The pulsed lamp was also measured with two radiometers (IL 1400 and IL 1700) with various bandpass filters. The measured fluence with the different filters is shown in Tables 1 and 2.

2.3 IVDS Description

The virus samples were analyzed using the IVDS. The detection stage of the IVDS eonsists of an electrospray unit to inject samples into the deteetor, a differential mobility analyzer (DMA), and a condensate partiele counter (CPC), shown in Figure 9.

The eleetrospray unit subjeets a eonduetive liquid to a strong electrie field. The field produces a cone that emits a fine jet that then breaks up into small droplets and forms a fine plume. To eliminate the possibility of the breakdown (corona discharge) of the air in the plume, as caused by the high electric field, the spray tip is surrounded by a flow of CO_2, which will prevent corona diseharge.

The DMA separates partieles by their eleetrieal mobility in air. The sample stream flows through a gap between a rod and a cylinder with an electrical potential between the two. Particle mobility, which is related to size and charge, will either pass particles through the DMA or impinge on the walls. With singly charged partieles, which are generated by the electrospray, the mobility becomes a direct measure of the particle size.

In the CPC, the sample partieles flow in tandem with a saturated working fluid of butanol. The nanosized partieles initiate the eondensation of the butanol, and the stream is then cooled. A standard optical counter can then count the butanol-condensed particles, and the results are displayed via the supplied software.

A complete description of the IVDS system, including the detector, can be found in ERDEC-TR-453.

3. RESULTS

IVDS Results

- MS2 Baeteriophage Unfiltered. The MS2 bacteriophage, #D4, was measured with the IVDS prior to each set of pulsed light exposures, when there was a new dilution from the stock sample. The wells of the microscope slide were filled with 100 µl of solution. For multiple exposures, the samples were left in the wells until the target number of pulses was reached. For example, if there was to be a sample with 10 pulses and another with 20 pulses, two wells would be filled. The two wells would then be pulsed 10 times with one well being emptied after pulsing. The remaining well would then be subjected to another 10 pulses, and the sample would be removed. The second well would then have received 20 pulses. Multiple well samples could then be compared as the same stock sample was exposed to differing pulse counts.

To determine the effects of the pulsed light on a sample of MS2 bacteriophage, #D4, the well slide was filled individually with 100 µl MS2 in each well. The stock sample of MS2 #D4, as analyzed with the IVDS, is shown in Figure 10. The slide was placed under the filter holder, without any filter. The slide was pulsed with 5 pulses each, and one well was removed after each pulse session, resulting in samples with 5, 10, 15, 20, 25, and 30 pulses each. The samples were analyzed with the IVDS, and the results are shown in Figure 11. The peak counts from the IVDS graph are shown in Table 3.

- MS2 GG400 Filter. Three wells in the well slide were filled with MS2 #D4 after its dilution from the stock sample. The new dilution is shown in Figure 12. The slide was placed under the filter holder with the GG400 filter in place. The samples were pulsed for 10, 20, and 30 pulses each. The IVDS analysis of the pulsed sample is shown in Figure 13. The top section of the IVDS scan is shown for clarity of defining the peak counts. The peak counts from the IVDS graph for the GG400 filter are shown in Table 4.

- MS2 WG320 Filter. Three wells in the well slide were filled with MS2 #D4 after its dilution from the stock sample. The slide was placed under the filter holder with the WG320 filter in place. The samples were pulsed for 10, 20, and 30 pulses each. The IVDS analysis of the pulsed sample is shown in Figure 14. The top section of the IVDS scan is shown for clarity of defining the peak counts. The peak counts from the IVDS graph for the WG320 filter are shown in Table 5.

- MS2 WG225 Filter. Three wells of the well slide were filled with MS2 #D4 after its dilution from the stock sample. The slide was placed under the filter holder with the WG225 filter in place. The samples were pulsed for 10, 20, and 30 pulses each. The IVDS analysis of the pulsed sample is shown in Figure 15. The top section of the IVDS scan is shown for clarity of defining the peak counts. The peak counts from the IVDS graph for the WG225 filter are shown in Table 6.

- MS2 UV Cold Mirror. Three wells in the well slide were filled with MS2 #D4 after its dilution from the stock sample. The new dilution is shown in Figure 16. The slide was placed under the filter holder with the UV cold mirror filter in place. The samples were pulsed for 10, 20, and 30 pulses each. The IVDS analysis of the pulsed sample is shown in Figure 17. The top section of the IVDS scan is shown for clarity of defining the peak counts. The peak counts from the IVDS graph for the UV cold mirror filter are shown in Table 7.

- MS2 IR Suppression. Three wells in the well slide were filled with MS2 #D4 after its dilution from the stock sample. The slide was placed under the filter holder with the IR suppression filter in place. The samples were pulsed for 10, 20, and 30 pulses each. The IVDS analysis of the pulsed sample is shown in Figure 18. The top section of the IVDS scan is shown for clarity of defining the peak counts. The peak counts from the IVDS graph for the IR suppression filter are shown in Table 8.

- TBSV Unfiltered. Three wells in the well slide were filled with TBSV after its dilution from the stock sample. The TBSV dilution is shown in Figure 19. The slide was placed under the filter holder without a filter in place. The samples were pulsed for 10, 20, and 30 pulses each. The IVDS analysis of the pulsed sample is shown in Figure 20. The peak counts from the IVDS graph for the unfiltered sample are shown in Table 9.

- TBSV WG225 Filter. Three wells in the well slide were filled with TBSV after its dilution from the stock sample. The slide was placed under the filter holder with the WG225 filter in place. The samples were pulsed for 10, 20, and 30 pulses each. The IVDS analysis of the pulsed sample is shown in Figure 21. The peak counts from the IVDS graph for the WG225 filter are shown in Table 10.

4. DISCUSSION

The pulsed light exposure reduced the IVDS counts of MS2 and TBSV. The more UV light available to the sample produced a greater reduction in counts. The WG225 filter allowed the most UV to pass and produced the largest reduction in counts except for the unfiltered pulsed light exposure.

The reduction in counts for 30 pulses did not always result in a linear reduction in counts. As shown in Figure 2, the well plate used for the virus solutions is not symmetrical. The wells are shifted off center. The large pulse counts, typically 30 pulses, were always in the wells that were closest to the edge of the well slide. As the well edge was right against the edge of the filter holder, there may be a shadowing effect from the holder that reduces the pulsed lamp exposure. This may explain the non-linearity of the higher pulse exposures not having an increasing reduction of counts in some cases. Further experimentation is warranted to determine if the shadowing has affected the results. Future experiments will have a symmetrical well plate for exposing the materials to the pulsed light.

5. CONCLUSIONS

The pulsed light exposure reduced the counts of MS2 and tomato bushy stunt virus. Analysis with the Integrated Virus Detector System showed that the higher the number of pulses from the light, the larger the reduction in counts in the sample. There was also a greater reduction in counts when the filters used allowed more UV light to penetrate to the sample. This does not indicate that reduced counts are attributed only to UV wavelengths. Another experiment may resolve this question. Results do indicate that the killing effect observed from the pulses is additive. This further indicates that hardy microbes could be killed by increasing either the power of the lamp or the number of pulses.

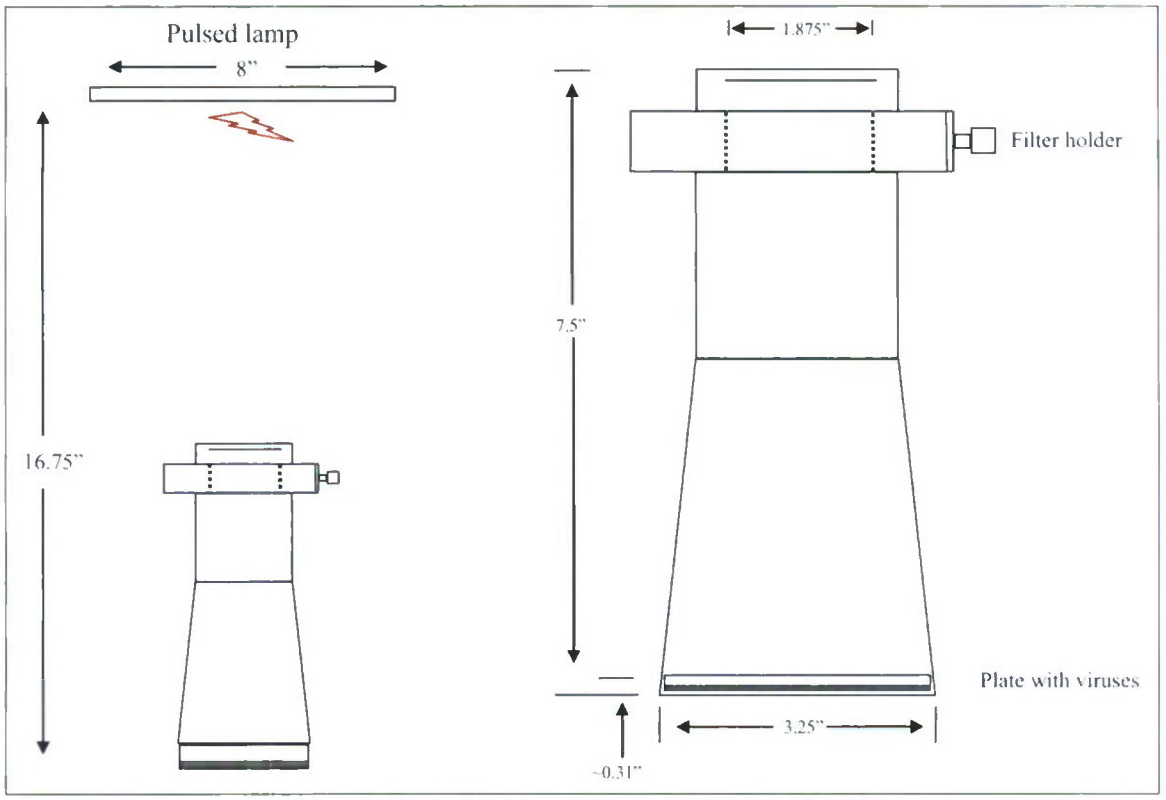

Figure 1. Pulsed lamp setup with filter hood

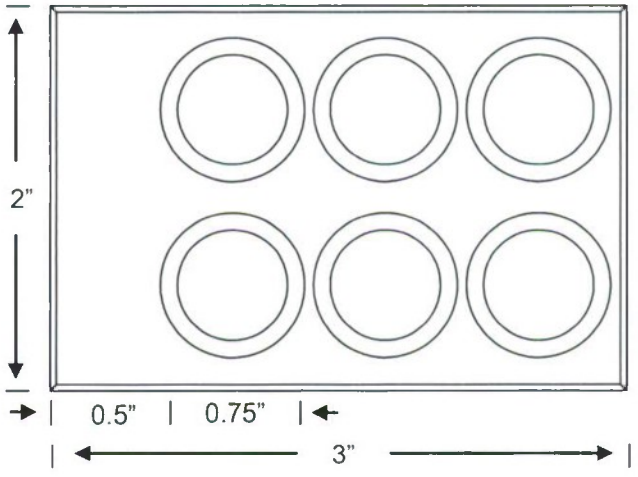

Figure 2. Virus holder for pulsed light exposure

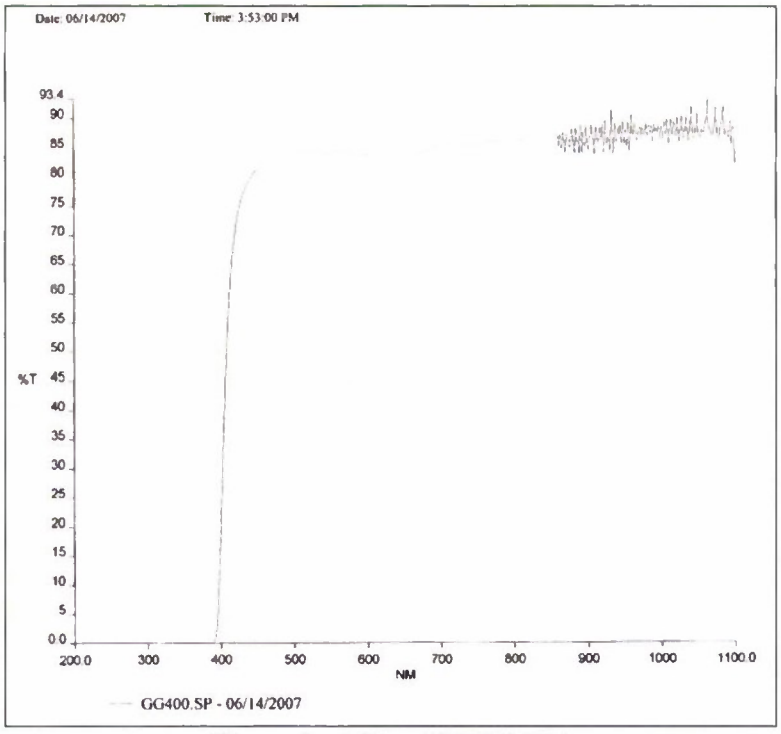

Figure 3. Filter GG400 %T

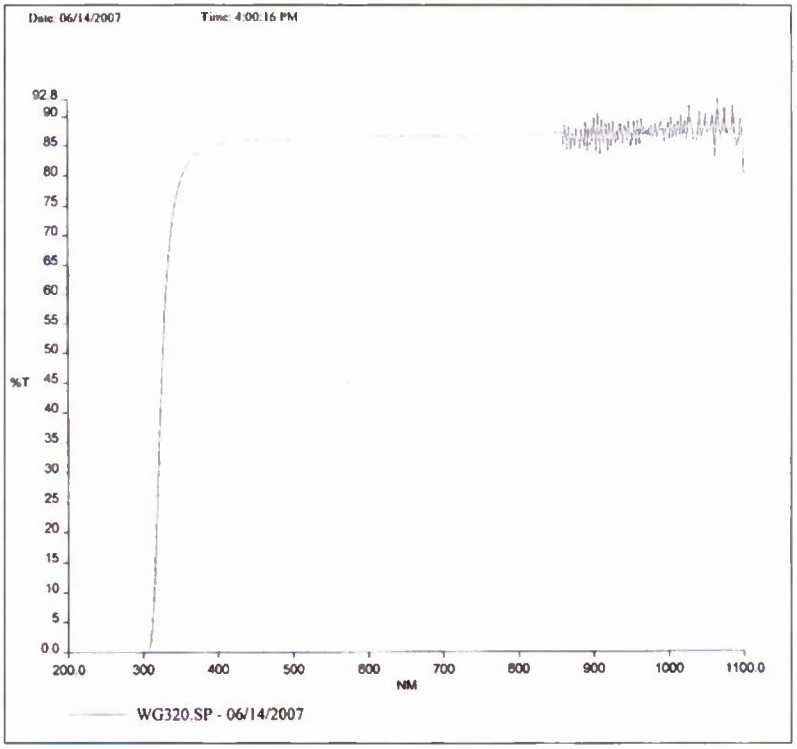

Figure 4. Filter WG320 %T

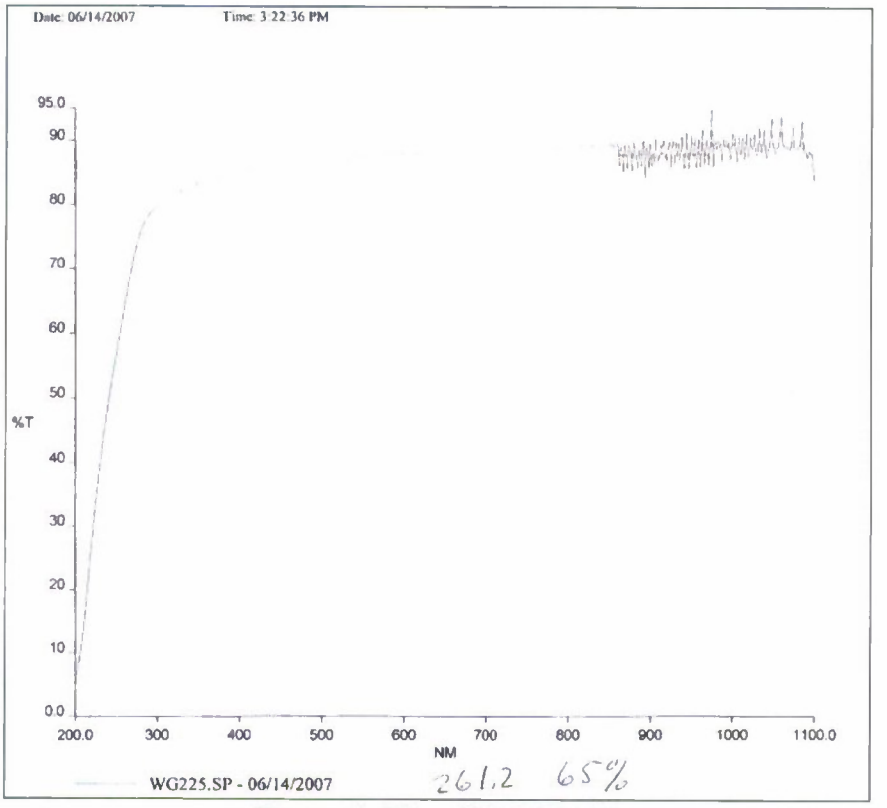

Figure 5. Filter WG225 %T

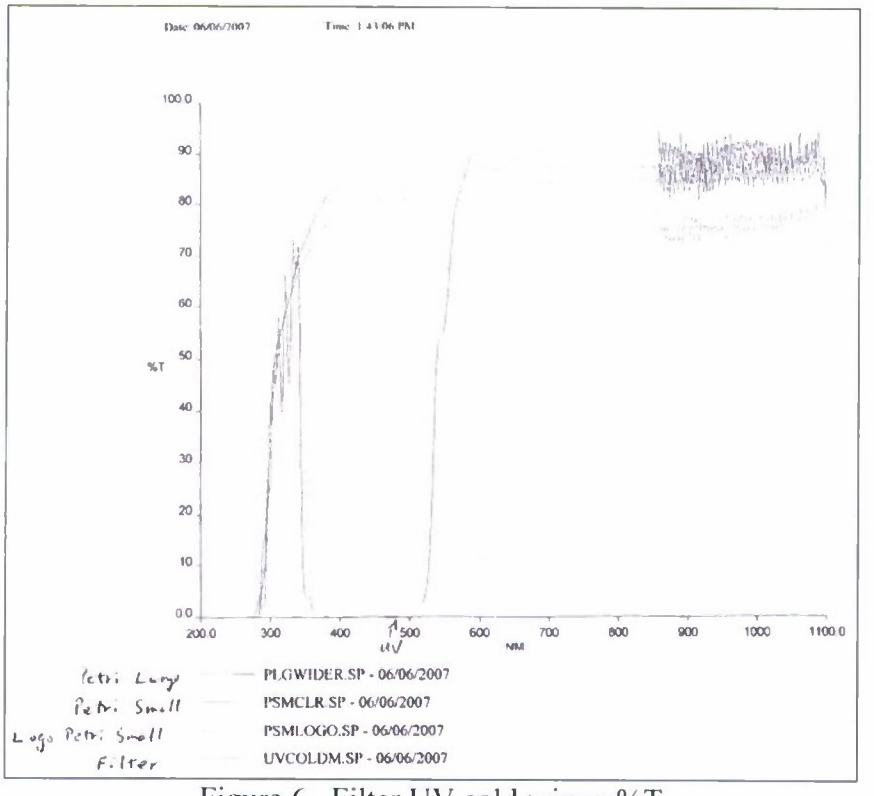

Figure 6. Filter UV cold mirror %T

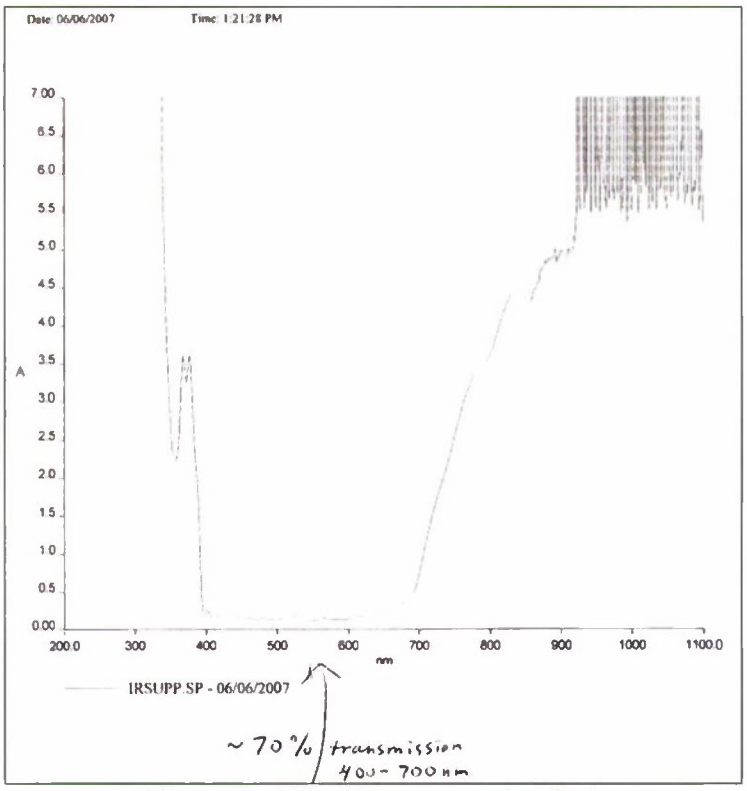

Figure 7. Filter IR suppression %A

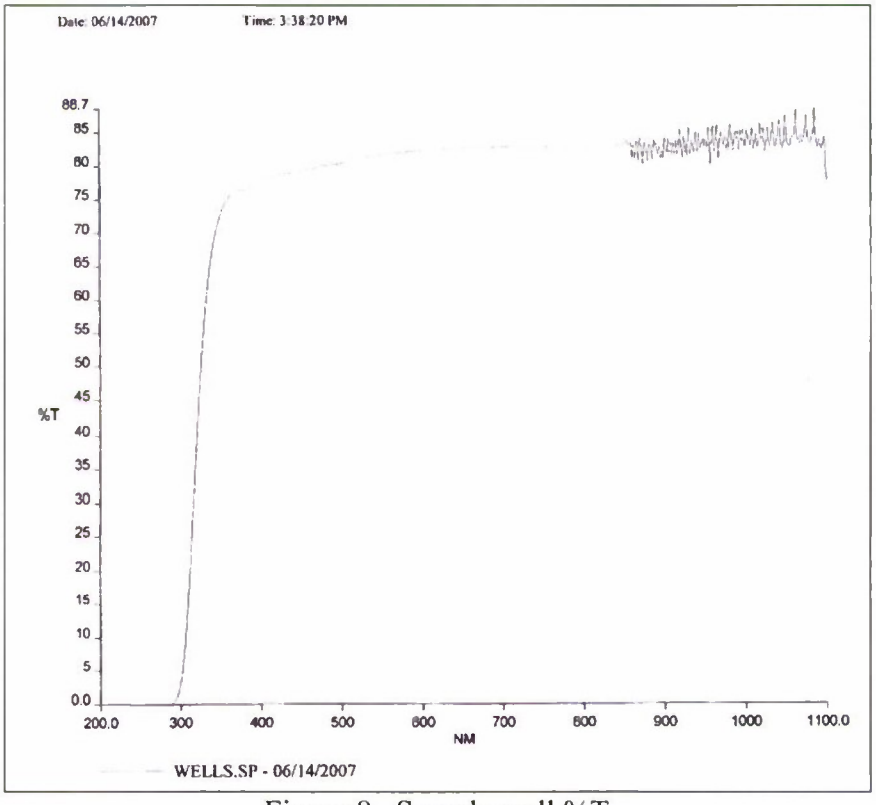

Figure 8. Sample well %T

Figure 9. IVDS analyzer

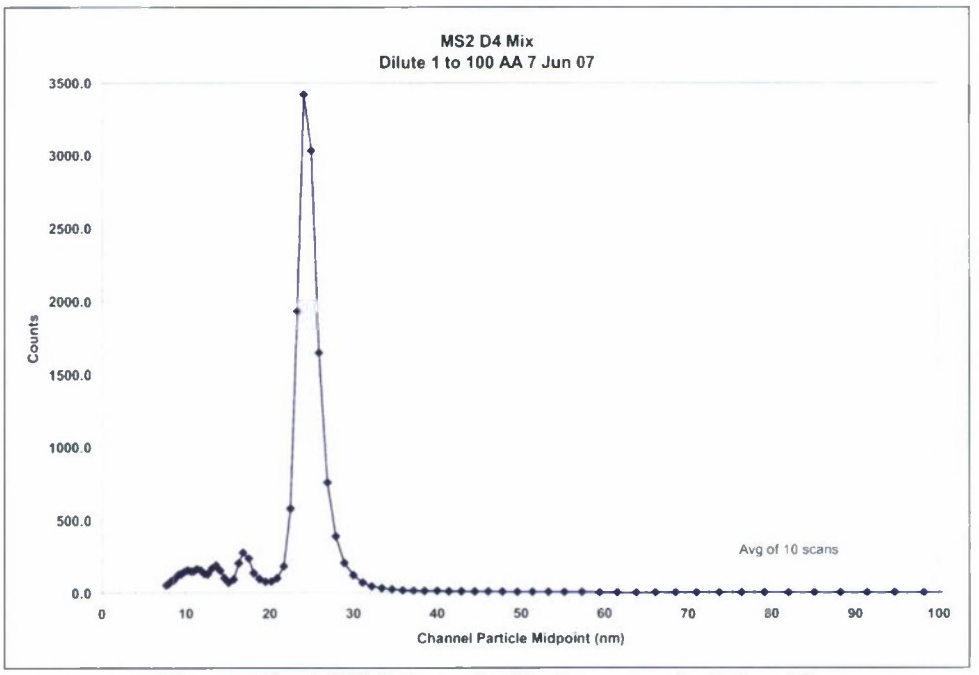

Figure 10. MS2 D4 stoek dilution sample 7 Jun 07

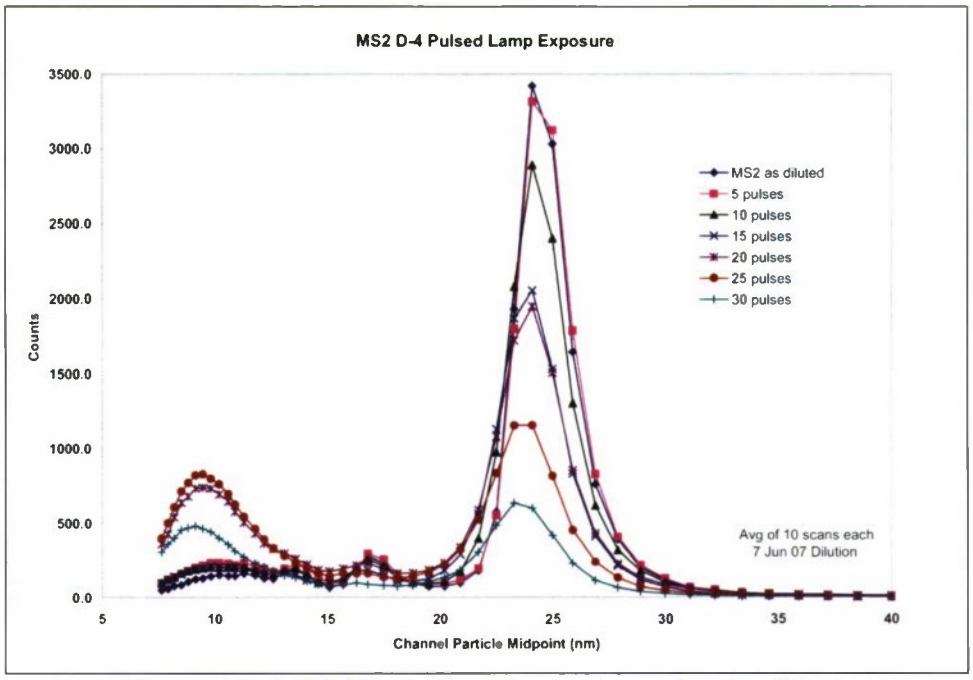

Figure 11. MS2 D4 stock and pulsed samples (no filter)

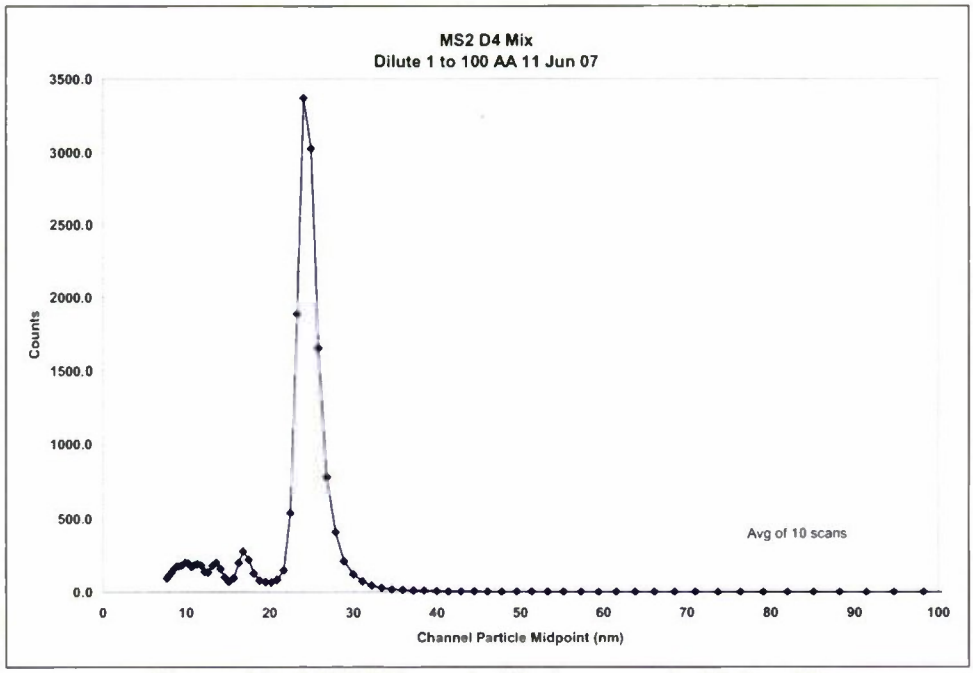

Figure 12. MS2 D4 stock dilution sample 11 Jun 07

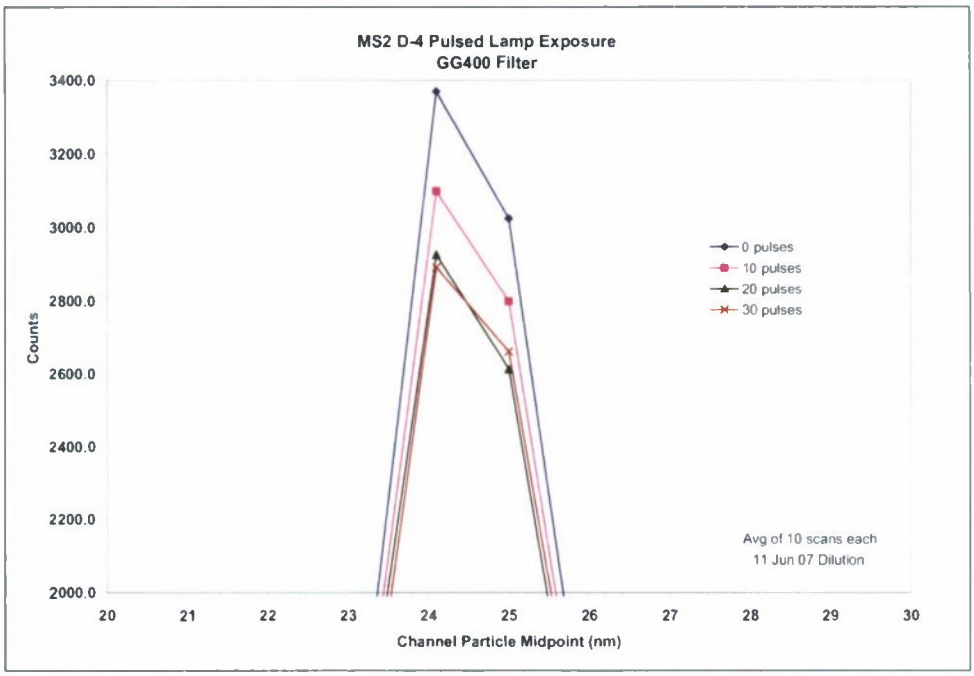

Figure 13. MS2 D4 stock and pulsed samples (GG400)

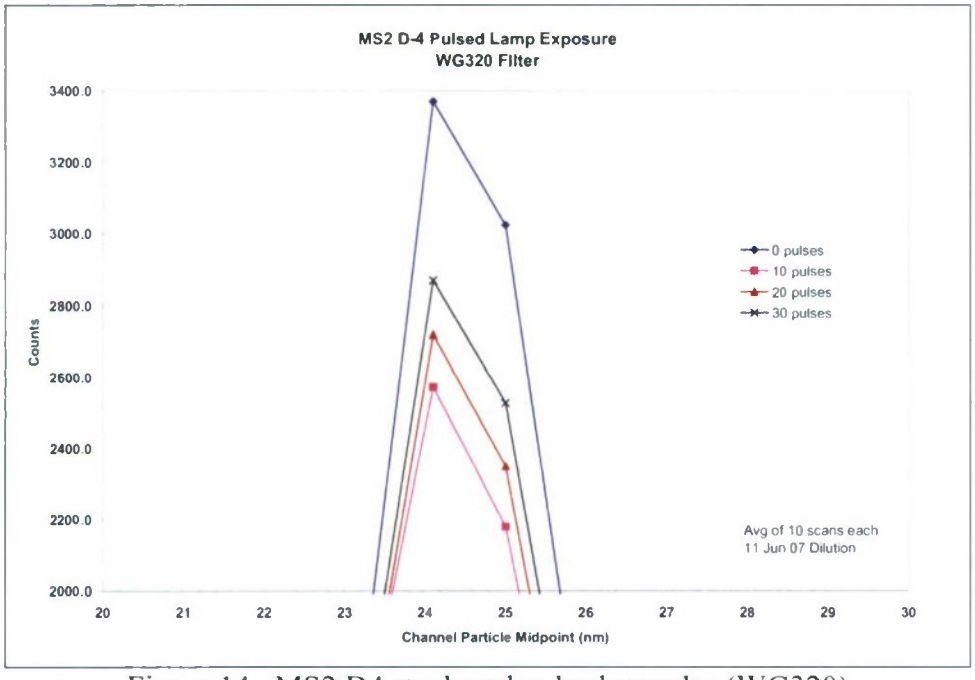

Figure 14. MS2 D4 stock and pulsed samples (WG320)

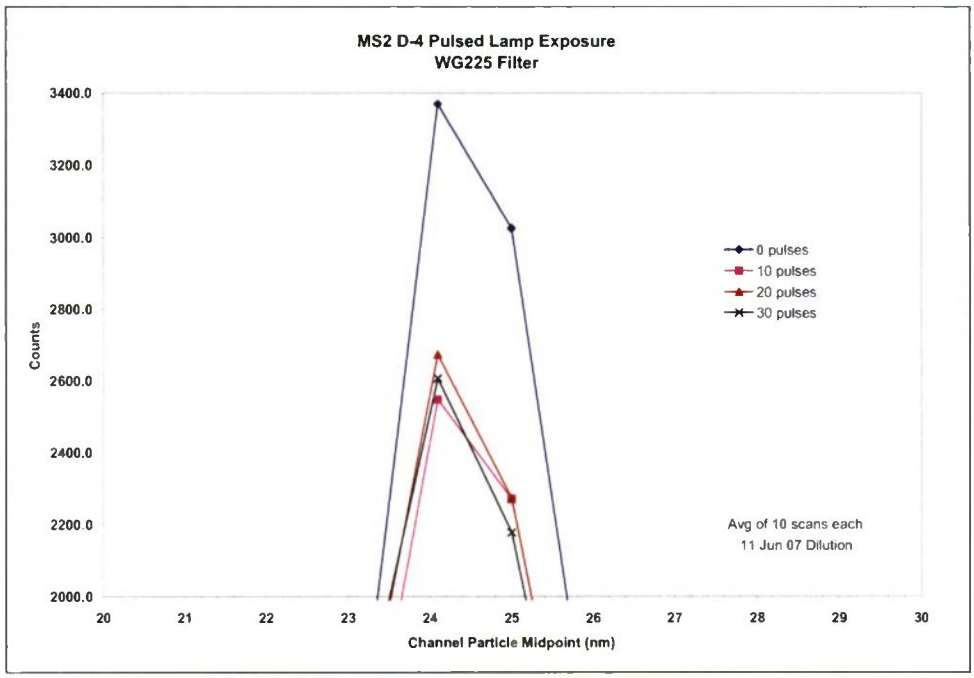

Figure 15. MS2 D4 stock and pulsed samples (WG225)

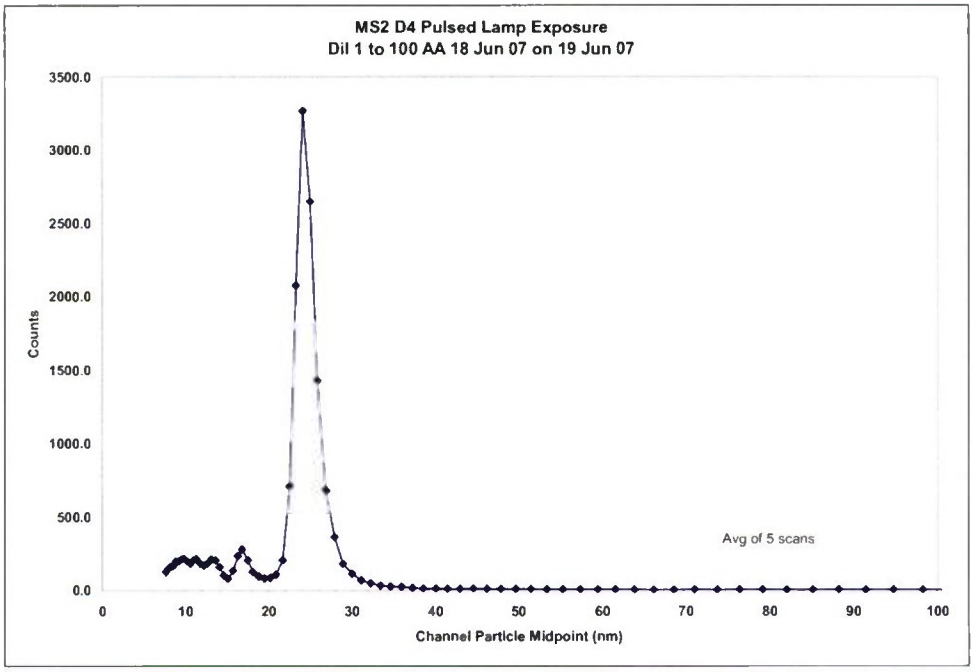

Figure 16. MS2 D4 stock dilution sample 19 Jun 07

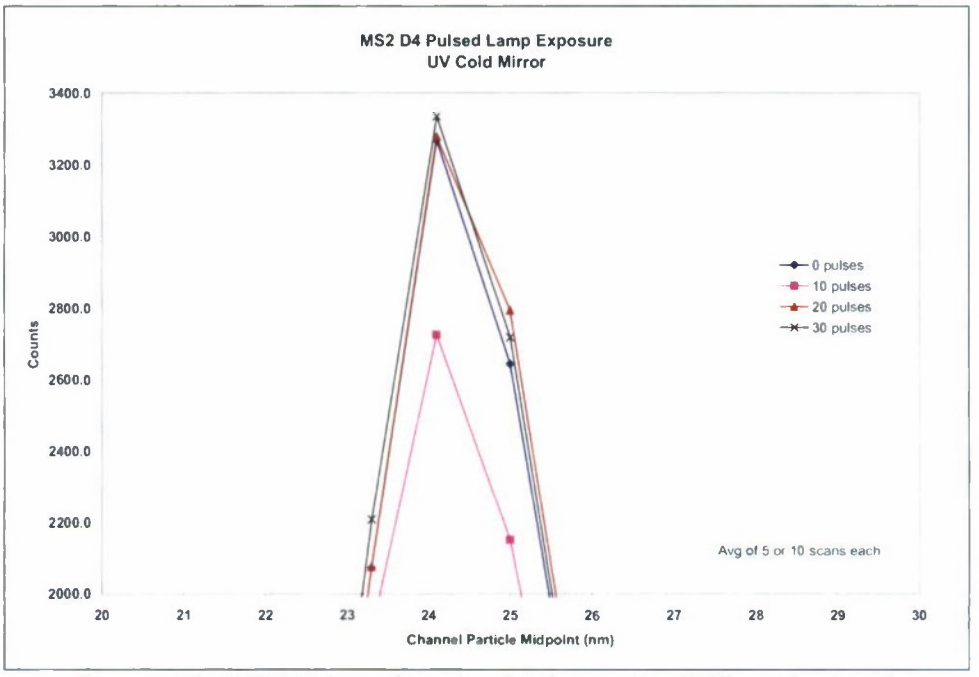

Figure 17. MS2 D4 stock and pulsed samples (UV cold mirror)

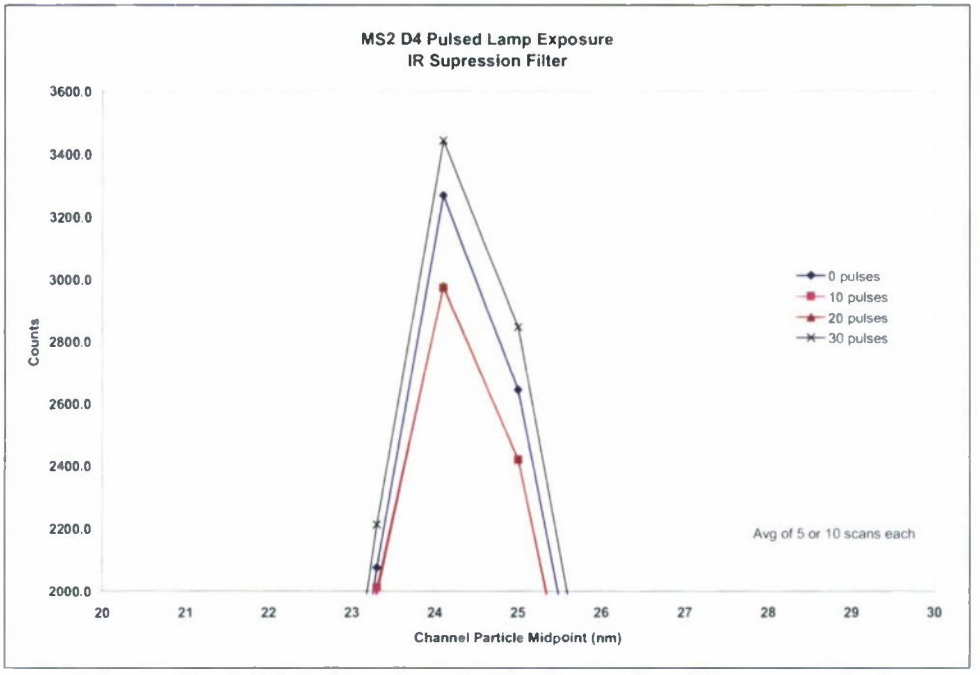

Figure 18. MS2 D4 stock and pulsed samples (IR suppression)

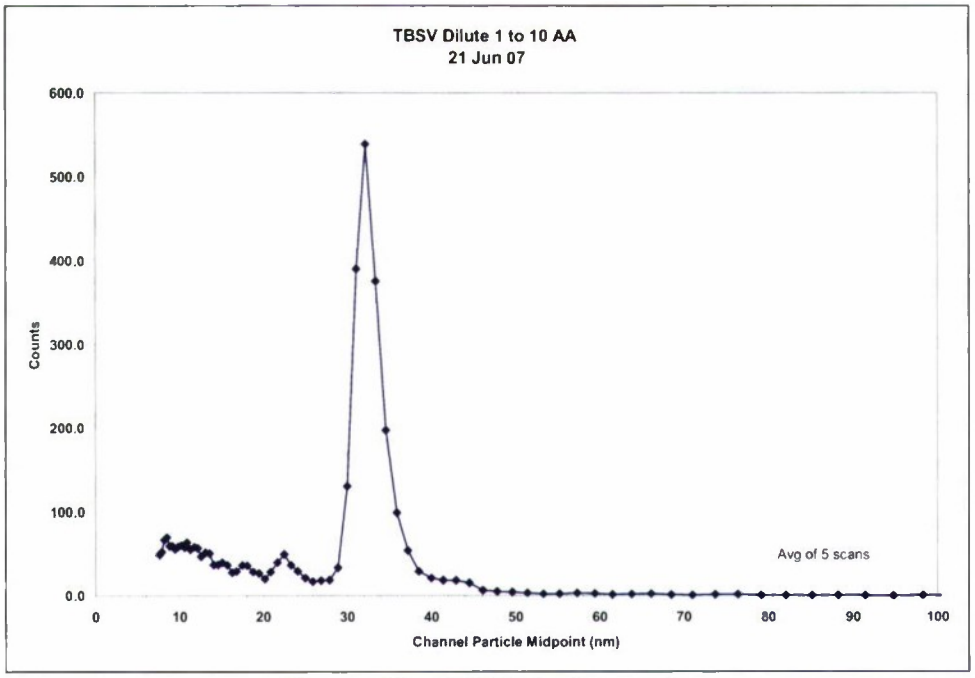

Figure 19. TBSV stock sample

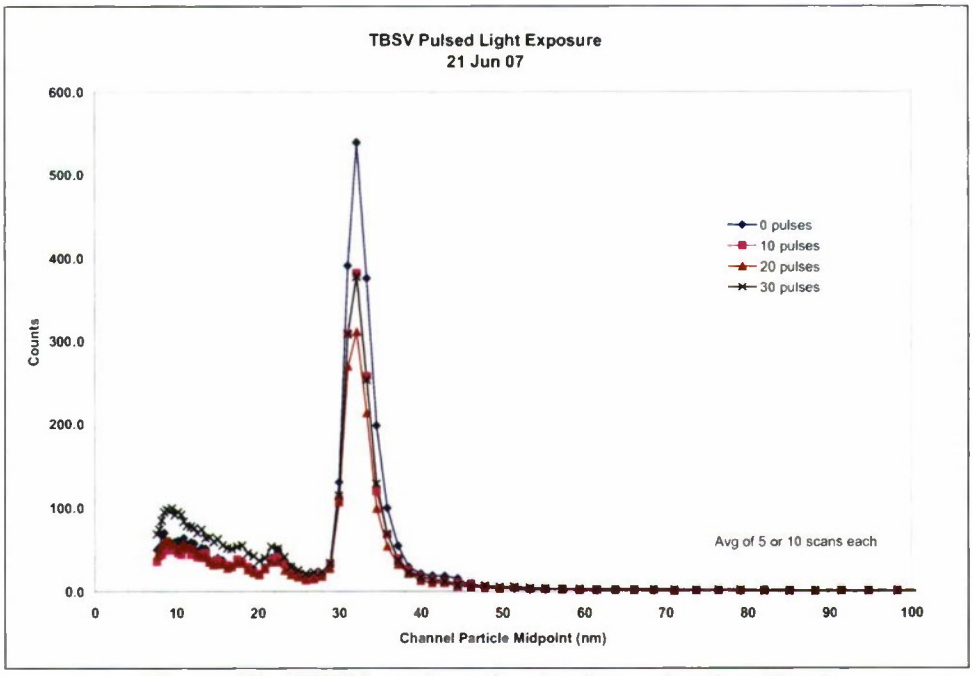

Figure 20. TBSV stock and pulsed samples (no filter)

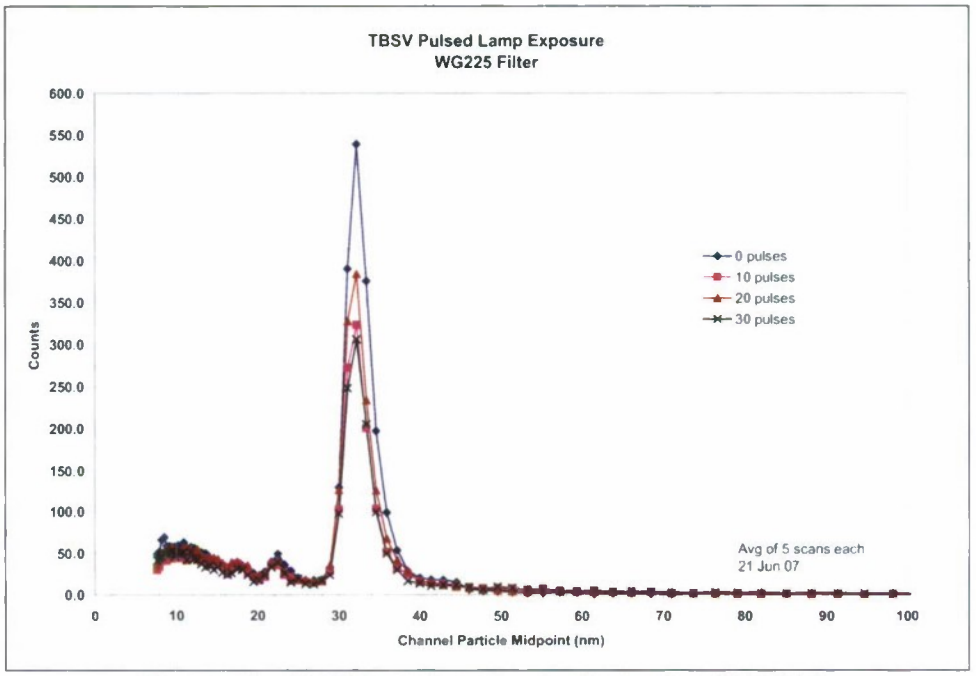

Figure 21. TBSV stock and pulsed samples (WG225)

Table 1. IL 1400 (Set B, SEL 623, S/N 647)

Filter	Orientation	Pulses	Fluence (mJ/cm^2)
NBP 400.2 nm		4	1.4
NBP 632.8 nm	Mirror up	3	1.9
NBP 632.8 nm	Mirror up	3	2.0

Table 2. IL 1700 (Set A, SED 623 S/N 180)

Filter	Orientation	Pulses	Fluence (mJ/cm^2)
NBP 400.2 nm		3	1.45
NBP 400.2 nm		3	1.45
NBP 400.2 nm	Filter reversed	3	1.49
NBP 254 nm	Mirror up	3	0.71
NBP 254 nm	Mirror up	3	0.66
NBP 254 nm	Mirror down	3	0.77
NBP 632.8 nm	Mirror up	3	1.95
NBP 632.8 nm	Mirror down	3	1.73
NBP 632.8 nm	Mirror down	3	1.7
NBP 632.8 nm	Mirror up	3	1.5
NBP 632.8 nm	Mirror up	3	1.82
NBP 632.8 nm	Mirror down	3	1.98

Table 3. Number of pulses and peak counts for MS2 D4 (No filter)

Pulses	Peak cts (7 Jun 07)	Reduction (%)
0.0	3420	-
5.0	3317	3
10.0	2892	15
15.0	2056	40
20.0	1951	43
25.0	1156	66
30.0	635	81

Table 4. Number of pulses and peak counts for MS2 D4 GG400 filter

Pulses	Peak cts GG400 Filter	Reduction (%)
0.0	3370	-
10.0	3100	8
20.0	2927	13
30.0	2892	14

Table 5. Number of pulses and peak counts for MS2 D4 WG320 filter

Pulses	Peak cts WG320 Filter	Reduction (%)
0.0	3370	-
10.0	2574	24
20.0	2720	19
30.0	2871	15

Table 6. Number of pulses and peak counts for MS2 D4 WG225 filter

Pulses	Peak cts WG225 Filter	Reduction (%)
0.0	3370	-
10.0	2549	24
20.0	2674	21
30.0	2607	23

Table 7. Number of pulses and peak counts for MS2 D4 UV cold mirror filter

Pulses	Peak cts UV cold mirror	Reduction (%)
0.0	3269	-
10.0	2726	17
20.0	3279	+0.3
30.0	3334	+2

Table 8. Number of pulses and peak counts for MS2 D4 IR suppression filter

Pulses	Peak cts IR suppression	Reduction (%)
0.0	3269	-
10.0	2974	9
20.0	2978	9
30.0	3443	+5

Table 9. Number of pulses and peak counts for TBSV (No filter)

Pulses	Peak cts unfiltered	Reduction (%)
0	539	-
10	381	29
20	311	42
30	378	30

Table 10. Number of pulses and peak counts for TBSV WG225 filter

Pulses	Peak cts WG225	Reduction (%)
0	539	-
10	324	40
20	384	29
30	306	43

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Wick, C.H.; Yeh, H.R.; Carlon, H.R.; Anderson, D. *Virus Detection: Limits and Strategies*; ERDEC-TR-453; U.S. Army Edgewood Research, Development and Engineering Center: Aberdeen Proving Ground, MD, 1997; UNCLASSIFIED Report (AD-A337 490).